

Applicants: Kmiec *et al.* Application No.: 09/818,875 Filed: March 27, 2001

For: TARGETED CHROMOSOMAL GENOMIC ALTERATIONS WITH MODIFIED SINGLE STRANDED OLIGONUCLEOTIDES

NaPro-4

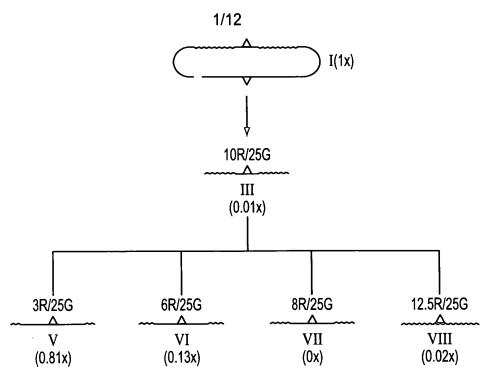


FIG. 1A

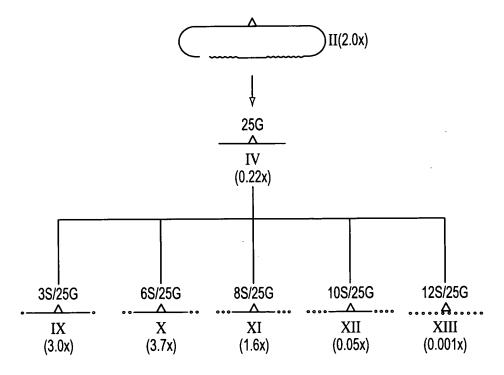
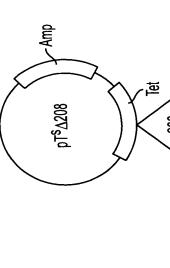


FIG. 1B

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: CONVERTED : MUTANT



pK<sup>S</sup>m4021

Ka an

4021

MUTANT: '------G' CONVERTED:-----C-WILD-TYPE:-----T-

TGCGCGaaggcugucgTA图CGgucagugauaT<sub>T</sub> T\_\_\_\_T

CCTCTCCGATAAGCCGATGCTGACCCGTGTT~

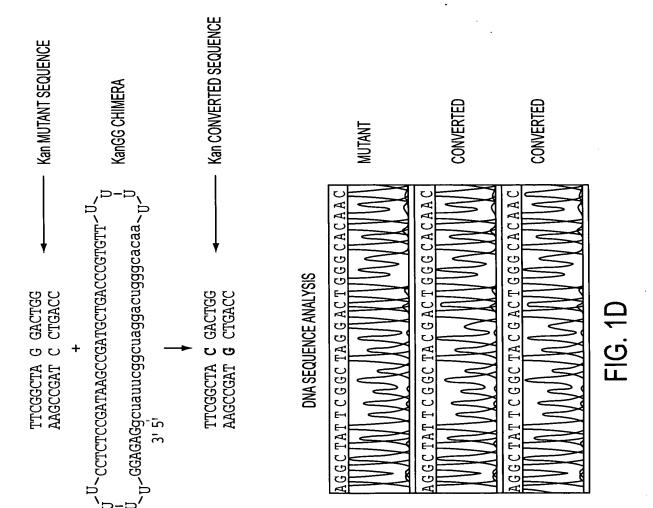
KanGG

Tet∆208T

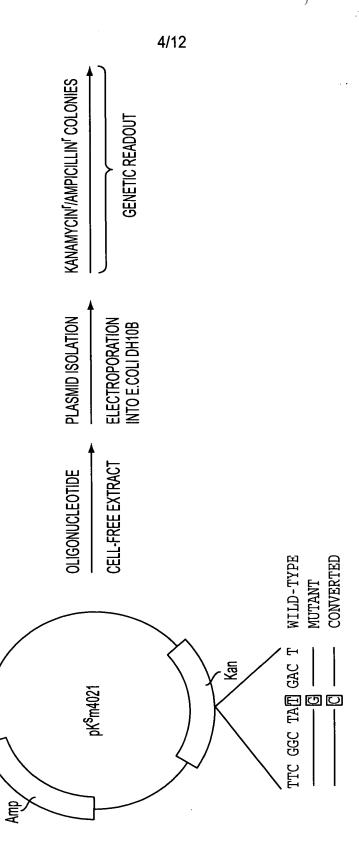
TCGCGC TTCCCACAGCATCGCCAGTCACTATT
3' 5'

FIG. 1C  $\mathcal{L}_{\mathrm{GGAGAC}}$ 

PLASMIDS, DNA TARGETS AND CHIMERIC OLIGONUCLEOTIDES

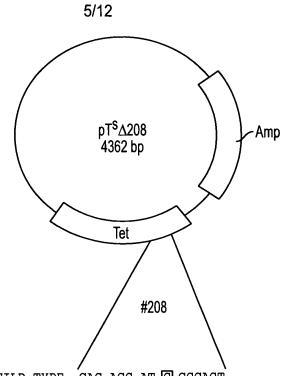






=1G. 2





WILD TYPE: GAC AGC AT C GCCAGT
MUTANT: GAC AGC AT - GCCAGT
CONVERTED: GAC AGC AT T GCCAGT

# SEQUENCE ANALYSIS OF $\mathsf{Tet}^\mathsf{f}$ PLASMID $\Delta 208$

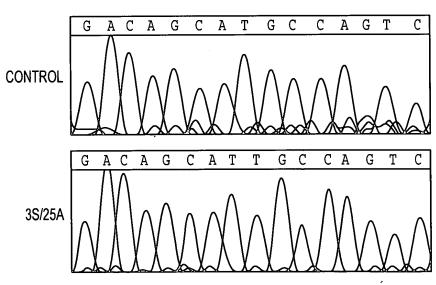


FIG. 3



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### DNA SEQUENCE ANALYSIS OF Kan<sup>r</sup> PLASMIDS

TARGET CODON E	DISTRIBUTION				
OLIGOMER	TAG	TAC	TAC/TAG	TGG	TCG
1) 3S/25G (20)		+			
2) 6S/25G (20)		+			
3) 8S/25G (20)		+			
4) 10S/25G (18)		+		+(2)	+(2)
5) 25S/25G (4)	•••		+(2)	+(2)	

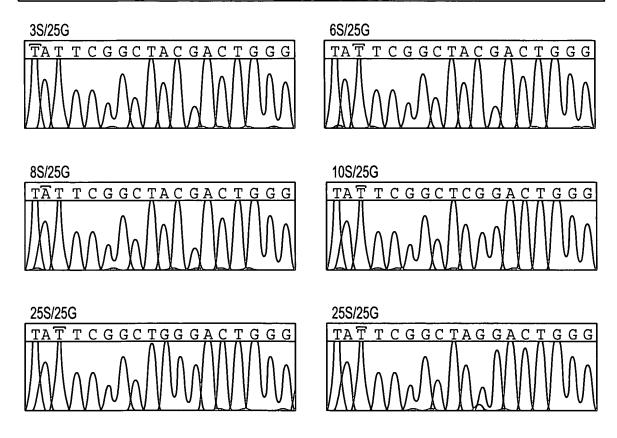


FIG. 4



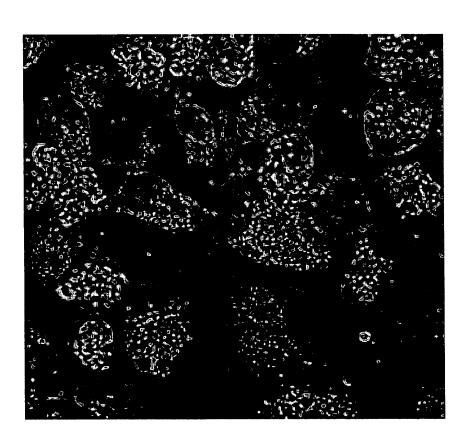


FIG. 5

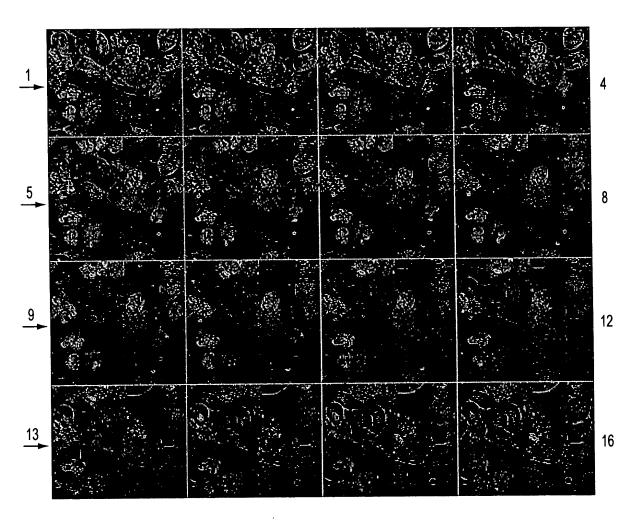
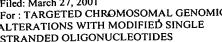
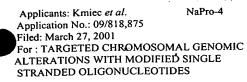


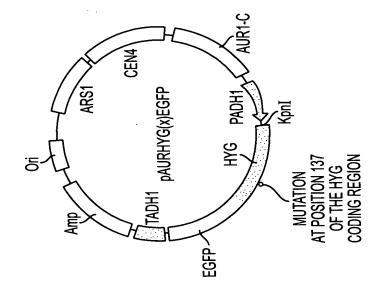
FIG. 6







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GTGGATAATGTCCT

GTGGATACGTCCT

GTGGATATGTCCT

SEQUENCE OF NORMAL ALLELE:

TARGET/EXISTING MUTANT:

DESIRED ALTERATION:

FIG. 7B

GTGGATAGGTCCT GTGGATACGTCCT TARGET/EXISTING MUTANT: DESIRED ALTERATION:

GTGGATATGTCCT

SEQUENCE OF NORMAL ALLELE:



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STRANDED OLIGONUCLEOTIDES

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HygE3T/25: 5'-AGG GCG TGG ATA CGT CCT GCG GGT A-3'

HygE3T/74: 5'-CTC GTG CTT TCA GCT TCG ATG TAG GAG GGC GTG GAT ACG TCC TGC GGG TAA ATA GCT GCG CCG ATG GTT TCT AC-3'

HygE3T/74α: 5'-GTA GAA ACC ATC GGC GCA GCT ATT TAC CCG CAG GAC GTA TCC ACG CCC TCC TAC ATC GAA GCT GAA AGC ACG AG-3'

## HygGG/Rev:

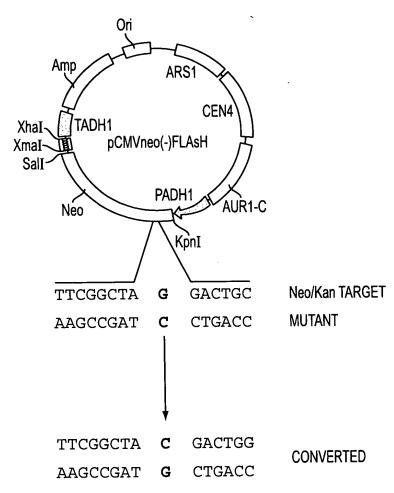
 $_{\rm T}$  ACATCCTCCCGCACCTATGCAGGACGCCCAT  $_{\rm T}$  $T_T$ TGTAGGagggcguggaTAGGTccugcgggua $T_T$ 

5'-CAT CAG AGC AGC CAA TTG TCT GTT GTG CCC AGT CGT AGC CGA ATA GCC TCT CCA CCC AAG CGG CCG GAG A-3'

FIG. 8



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#### FUSION GENE FOR LIGAND BINGING

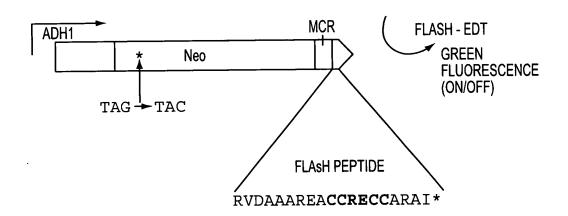


FIG. 9

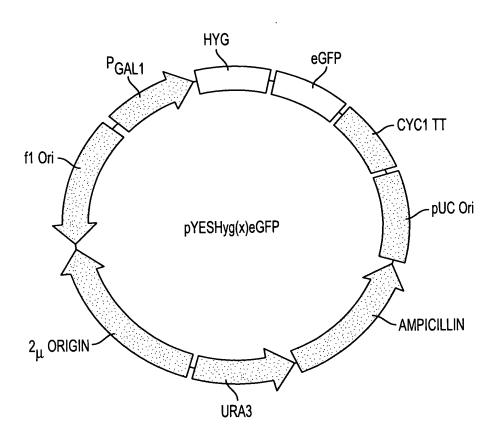


FIG. 10